

IN THE CLAIMS

1. (currently amended) An integrated microfluidic sperm isolation and insemination device, comprising:

- a) at least two ~~gravity~~ pump liquid reservoirs, one of said reservoir being a sperm receiving reservoir, one of said reservoirs being a sort media liquid reservoir;
- b) at least one sort channel, said sort channel having a sort side and a reject side, and an upstream portion in fluid communication with said sperm receiving reservoir on said reject side of said sort channel, and in fluid communication with at least one sort media reservoir on said sort side of said sort channel;
- c) a rejected sperm receiving reservoir in fluid communication with said downstream portion of said sort channel on said reject side of said sort channel;
- d) a sorted sperm reservoir in fluid communication with ~~said a~~ downstream portion of said sort channel on said sort side of said sort channel, and
- e) an oocyte insemination chamber sized to contain one or more oocytes and into which sorted sperm flows, said oocyte insemination chamber configured to contain at least one barrier which prevents egress of oocyte(s) located in said chamber but which is configured to allow fluid flow into or through said chamber, wherein said oocyte insemination chamber and said sorted sperm reservoir may together be a single chamber, and wherein sperm-containing liquid in said sperm receiving reservoir and a sort media liquid in a second reservoir exhibit gravity induced co-laminar flow of both liquids through said sort channel in parallel but separate streams having an interface there between, wherein motile sperm cross said interface and are ultimately transported in said sort media liquid to said oocyte insemination chamber from said sort channel or from said sorted sperm reservoir.

2. (original) The device of claim 1, constructed of a silicone elastomer.

3. (original) The device of claim 1, having an oocyte duct communicating with said oocyte insemination chamber.

4. (original) The device of claim 1, wherein said sorted sperm reservoir and said oocyte insemination chamber are a single chamber, also in communication with an oocyte duct.

5. (original) The device of claim 1, wherein said barrier comprises a three dimensional barrier grate having forming plurality of barrier flow channels, said barrier flow channels smaller than an oocyte diameter by an amount such that said oocyte is blocked from passing through said barrier flow channels, but larger than the size of sperm such that sperm may travel through said barrier.

6. (original) A method for in vitro insemination of an oocyte with sorted, motile sperm with minimal manipulation of said oocyte, said method comprising

- a) selecting a device of claim 1;
- b) introducing one or more oocytes into said oocyte insemination chamber;
- c) introducing a first sperm-containing liquid to be sorted into a second sperm-containing liquid containing sperm of higher average motility than sperm of said first sperm containing liquid;
- d) introducing a sort media liquid into said sort media liquid reservoir;
- e) flowing said first sperm-containing liquid and said sort media liquid co-laminarly through said sort channel;
- f) removing from said sort channel said second sperm-containing liquid; and
- g) contacting said second sperm-containing liquid with said oocyte(s).

7. (original) The method of claim 6, wherein said oocyte insemination chamber communicates with an oocyte duct, said method further comprising introducing said oocyte(s) into said oocyte insemination chamber through said oocyte duct, and removing one or more sperm-contacted oocytes from said oocyte insemination chamber through said oocyte duct.

8. (original) The method of claim 7, wherein said sperm-contacted oocyte is a fertilized oocyte.

9. (original) The process of claim 7, wherein introducing and removing of said oocyte(s) from said oocyte insemination chamber are performed with a pipet.

10. (currently amended) The method of claim 6, wherein said oocyte insemination chamber is configured such that a plurality of oocytes occupying said oocyte insemination chamber are forced to assume serial positions with respect to the direction of fluid flow.

11. (currently amended) The method of claim 6, wherein one or more oocytes are fertilized by sperm in said second sperm-containing liquid, and a growth media liquid is introduced into at least one of said ~~gravity~~ pump liquid reservoirs to provide a flow of growth media past said fertilized oocyte(s).

12. (currently amended) A method for improving ~~[[the]]~~ rate of fertilization of oocytes when employing low sperm concentration, comprising introducing one or more oocytes into a microfluidic channel, said microfluidic channel having disposed therein a barrier having openings therein through which liquid and sperm may flow, but which are too small to allow an oocyte to enter; introducing liquid and causing said oocyte(s) to travel through said microfluidic channel to said barrier; introducing a sperm-containing liquid of low sperm concentration into said microfluidic channel and flowing said sperm-containing liquid past said oocyte(s), wherein ~~[[the]]~~ said rate of fertilization at ~~[[the]]~~ said low sperm concentration used is higher than the rate achieved in center-well

fertilization.

13. (original) The method of claim 12, wherein the sperm concentration is less than 0.5×10^6 sperm/mL.